Docket No. 60388-A-PCT-US/JPW/GJG/JBC

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Mary Cismowski and Emir Duzic

Serial No.: 09/709,103 Examiner: D.M. Sullivan

Filed : November 8, 2000 Group Art Unit: 1636

For : AGS PROTEINS AND NUCLEIC ACID MOLECULES AND USES THEREFOR

1185 Avenue of the Americas
New York, New York 10036

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SIR:

DECLARATION UNDER 37 C.F.R. §1.131

We, Mary Cismowski and Emir Duzic, hereby declare as follows:

1. We are named as coinventors on the above-identified patent application.

2. We understand that the invention is recited in claims 81-93 as amended by the accompanying amendment. The text of claims 81-93 is attached hereto as **Exhibit 1**.

3. We conceived of the invention recited in claims 81-85 and 87-93 in the United States prior to March 31, 1998.

4. We also, either personally or through persons working under our direction and supervision, reduced to practice prior to March 31, 1998 a nucleic acid comprising nucleotides having a sequence which encodes an Activator of G Protein Signaling ("AGS") protein, a vector comprising the nucleic acid, and a host cell containing the vector and expressing the AGS protein.

5. As evidence of the fact that we, either personally or through persons working under our direction and

supervision, reduced to practice prior to March 31, 1998 a nucleic acid comprising nucleotides having a sequence encoding an AGS protein, a vector comprising the nucleic acid, and a host cell containing the vector and expressing the AGS protein, we attach hereto as **Exhibit 2** a copy of a page entitled "Test of UK w/Various Strains + [AT]" and as **Exhibit 3** a copy of a page entitled "Creation of Point Mutations of UK," both from the laboratory notebook of Mary Cismowski. The dates have been deleted from Exhibit 2 and Exhibit 3. All of the deleted dates were prior to March 31, 1998.

6. The copy of the page submitted as **Exhibit 2** shows the expression of a protein named "UK" by various strains of cells into which a nucleic acid comprising nucleotides having a sequence encoding the protein named "UK" was inserted. The nucleic acid comprised nucleotides having a sequence as set forth in SEQ ID NO:1 of the subject application.

7. The copy of the page submitted as **Exhibit 3** shows that the amino acid sequence of the protein named "UK" is identical to the amino acid sequence set forth in SEQ ID NO:2 of the subject application, which represents the amino acid sequence of an AGS protein.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made herein on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Dated: August 21, 2003

Mary Cismowski, Ph.D.

Dated: _____

_____
Emir Duzic, Ph.D.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made herein on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Dated: _____

Mary Cismowski, Ph.D.

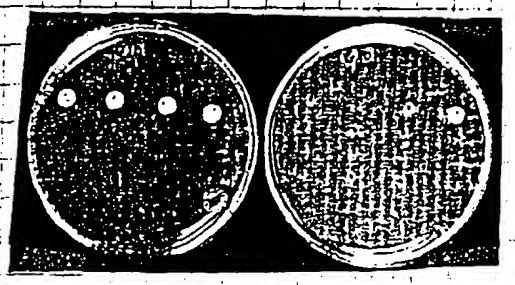
Dated: 08/22/03

Emir Duzic

Emir Duzic, Ph.D.

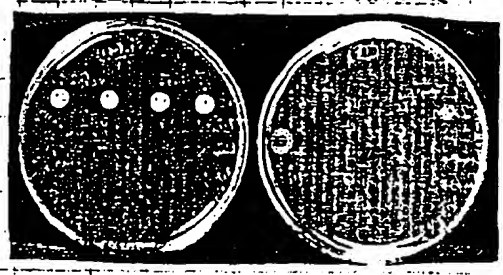
Project No._____

TEST OF UK W/ VARIOUS STRAINS + [AT]



10 mM AT

20 mM AT



Conclusions:

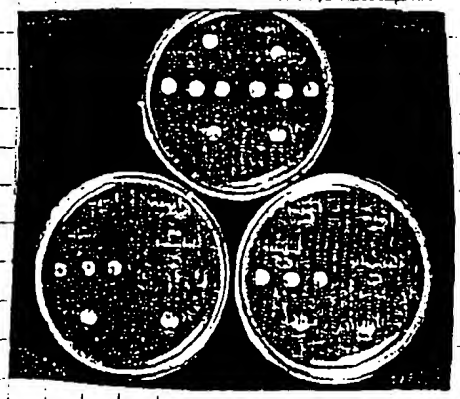
1. Gpa1(1-41)/Gai2 does not couple Ga as well as Gpa1 or the Gpa1(1-41)/Gai2 G204A mutant (see Glucose 0 plates)

2. The presence of the UK insert confers growth on all Ga strains relative to pYES2. This growth is galactose dependent - presumably is due to expression of UK.

3. Expression of UK, however, seems to be inhibiting growth of 1316/1127 relative to pYES2. This was not seen before - why?

4. Also, note that one of the two 1316/4098 isolates does exhibit lower growth than 1316/1183 when UK is induced. This may indicate an effect on Ga. May have to repeat this.

- Effect of Ste5Δ:



GLUCOSE-URA+ 1mM AT

SUCROSE-URA

GAL-URA + 1 mM AT

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Witnessed & Understood by me,

Invented by Mary Cairns

Recorded by

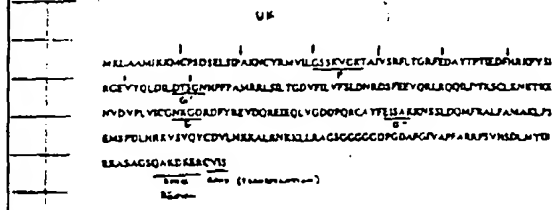
Date

Project No._____

BOOK NO._____ TITLE CREATION OF POINT MUTANTS OF UK

From Page No.__

-- UK looks like a ras-related G protein. It seems to have most of the conserved regions in small G proteins. Therefore, it may be possible to create "activating" + "inactivating" mutations of UK, as has been done with other small + heterotrimeric G proteins.



- The ras G12V activating mutation is in sequence ...GAG G V GCK SAL... in P region. UK does not have this G, but has Z in the P region (at acc. 3 + 36). These will be mutated to valine to see if one of them is an activating mutation.

- Glycine 81 in UK in the G' region is the equivalent of the G60 in Gαs. Mutation of this to alanine should produce an inactive UK protein.

- Jeff designed the following mutagenic oligo pairs + performed the mutagenesis. He used the pcDNA3.1-His-UK plasmid as parent. UK can be excised with BamHI + EcoRI. Jeff confirmed mutations by sequencing + gave me both one isolate for each mutation.

G31 → V:

Analysis of "G-V1 FWD" a 31-mer DNA Oligonucleotide

5' CGC ATG GTC ATC CTC GTT TCG TCC AAG GTG G 3'

Oligonucleotide Analysis *Analysis Parameters*

Analysis of "G-V1 REV" a 31-mer DNA Oligonucleotide

5' CCA CCT TGG ACG AAA CGA GGA TGA CCA TGC G 3'

Oligonucleotide Analysis *Analysis Parameters*

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Witnessed & Understood by me,

Invented by Mary Cowan

Recorded by

Date